

Claims:

We claim:

1. An apparatus for detecting particles on a substrate having a light-receiving
5 edge, the apparatus comprising in combination:
 - a substrate holder;
 - a processor;
 - a memory in communication with the processor;
 - an imaging module in communication with the processor, the imaging module
10 having a fixed position relative to the substrate holder;
 - an illumination module for illuminating the substrate by illuminating the light-receiving edge with light to create total internal reflection within the substrate; and
 - a set of instructions stored in the memory and executable by the processor to receive input from the imaging module and to provide an output indicating whether
15 particles are detected.
2. The apparatus of claim 1, wherein the imaging module and substrate holder are greater than 30 mm from one another and less than 356 mm from one another.
- 20 3. The apparatus of claim 2, wherein the imaging module comprises a photosensor and wherein the photosensor is less than 70 mm from the substrate holder.
4. The apparatus of claim 1, wherein the processor, memory device, imaging
25 module, substrate holder, illumination module, output module, and input module are contained within one housing.
5. The apparatus of claim 1, wherein the nanoparticles have been amplified with chemical signal amplification.
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6. The apparatus of claim 1, wherein the memory device includes a compensation module, the processor accessing the compensation module to compensate for distortion in an image acquired by the imaging module.

5 7. The apparatus of claim 6, wherein the compensation module compensates for grayscale distortion.

8. The apparatus of claim 6, wherein the compensation module compensates for spatial distortion.

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9. The apparatus of claim 1, wherein the memory device includes a program configured to perform the steps of:

acquiring multiple images by the imaging module of a substrate in the substrate holder, the substrate having at least one test spot containing a test sample
15 and at least another spot that is a control or a second test spot, the multiple images being taken at different exposures; and

determining, based on the multiple images of the spots, the presence of metallic nanoparticle complexes in the one test spot as an indication of presence of one or more of target analytes.

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10. The apparatus of claim 9, wherein the step of determining the presence of said metallic nanoparticle complexes in the spot containing the test sample comprises:

performing regression analysis on portions in the multiple images containing the one test spot and the control or second test spot to generate functions of exposure
25 time versus intensity for each of the spots;

selecting an exposure time;

determining intensity for the one test spot and the control or second test spot for the selected exposure time based on the functions generated; and

determining whether the one test spot containing the test sample contains
30 metallic nanoparticle complexes based on comparing the intensity of the one test spot with the intensity of the control or second test spot at the selected exposure time.

11. The apparatus of claim 10, wherein the selected exposure time is an optimal exposure time.
- 5 12. The apparatus of claim 1, wherein the memory device includes a program configured to perform the steps of:
- automatically detecting spots on substrate in the substrate holder, the substrate having a plurality of wells; and
 - automatically determining the wells based on the automatic detection of at
- 10 least a portion of the spots.
13. The apparatus of claim 12, wherein the step of automatically determining the wells comprises:
- automatically determining spacing between at least some of the detected spots;
- 15 and
- automatically determining spots which are located within at least one well based on the spacing.
14. The apparatus of claim 12, wherein the step of automatically determining the
- 20 wells comprises:
- automatically determining patterns for at least a portion of the spots detected;
 - and
 - automatically comparing the patterns with predetermined patterns for wells.
- 25 15. In a substrate having a light-receiving edge and a plurality of spots containing specific binding complements to one or more target analytes, at least one of the spots is a test spot for metallic nanoparticles complexed thereto in the presence of one or more target analytes, another spot is a control spot or a second test spot for metallic nanoparticles, with or without signal amplification, complexed thereto in the presence
- 30 of a second or more target analytes, a method for detecting the presence or absence of

the one or more of the target analytes in the test spot, the method comprising the steps of:

illuminating the light-receiving edge of the substrate to create total internal reflection within the substrate to illuminate the surface of the substrate;

5 acquiring multiple images of the test spot and the control or second test spot, the multiple images being taken at different exposures; and

determining the presence of said metallic nanoparticle complexes in the test spot as an indication of the presence of one or more of the target analytes based on the acquired multiple images of the spots.

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16. The method of claim 15, wherein the control spot is selected from the group consisting of metallic nanoparticle conjugated directly to the substrate via a nucleic capture strand, metallic nanoparticles printed directly on the substrate, and a positive result of metallic nanoparticles complexed to a known analyte placed in a separate
15 well.

17. The method of claim 15, wherein the test sample is a nucleic acid from a wildtype nucleic acid sequence; and

20 wherein the comparison sample is a nucleic acid from a mutant nucleic acid sequence that is related to the wildtype nucleic acid sequence.

18. The method of claim 15, wherein the substrate includes a plurality of wells, at least one of the wells containing the test and comparison spots;

25 further comprising the step of determining an optimal exposure time for the well; and

wherein the images acquired are taken at the optimal exposure time and at least one exposure time which is less than the optimal exposure time.

19. The method of claim 18, wherein the step of determining an optimal exposure
30 time comprises determining an exposure time which results in a predetermined saturation of the image acquired.

20. The method of claim 15, wherein the step of determining the presence of said metallic nanoparticle complexes in the spot containing the test sample comprises:
- performing regression analysis on the portions in the multiple images
5 containing the test and comparison spots to generate functions of exposure time versus intensity for each of the spots;
selecting an optimal exposure time;
determining intensity for the test and control spots for the optimal exposure time based on the functions generated; and
10 determining whether the test spot containing the test sample contains metallic nanoparticle complexes based on comparing the intensity of the test spot with the intensity of the comparison spot at the optimal exposure time.
21. The method of claim 20, wherein the image acquired results in pixels assigned
15 for the comparison and test spots, the pixels having pixel values;
wherein the step of performing a regression analysis comprises performing a regression analysis on the pixel values in the comparison and test spots.
22. The method of claim 21, wherein the step of selecting an optimal exposure
20 time comprises determining an exposure time which results in a predetermined saturation of a portion of the image acquired which contains the test and comparison spots.
23. The method of claim 22, wherein the step of determining intensity for the test
25 and comparison spots for the optimal exposure time based on the functions generated comprises interpolating or extrapolated the functions generated.
24. The method of claim 23, wherein the step of comparing the intensity of the
test spot with the intensity of the control spot at the optimal exposure time comprises
30 performing statistical analyses on the intensity of the comparison and test spots to

determine if the intensity of the test spot is similar or dissimilar to the comparison spot.

25. The method of claim 24, wherein the step of performing statistical analyses
5 comprises performing differences between means testing.

26. In a substrate having a light-receiving edge and a plurality of spots containing
specific binding complements to one or more target analytes, at least one of the spots
is a test spot for metallic nanoparticles, with or without signal amplification,
10 complexed thereto in the presence of one or more target analytes, another spot is a
control spot or a second test spot for metallic nanoparticles complexed thereto in the
presence of a second or more target analytes, an automatic method of detecting the
plurality of spots comprising the steps of:

illuminating the light-receiving edge of the substrate to create total internal
15 reflection within the substrate to illuminate the surface of the substrate;

acquiring at least one image of the plurality of spots composed of metallic
nanoparticles on the surface of the substrate;

compensating for at least one type of distortion in the acquired image; and
automatically determining locations of at least some of the plurality of spots
20 composed of metallic nanoparticles based on the compensated acquired image.

27. The method of claim 26, the step of acquiring being performed by an image
module which is less than or equal to 356 mm distance from the surface of the
substrate.

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28. The method of claim 27, wherein the image acquired by the image module
includes all or substantially all of the surface of the substrate.

29. The method of claim 27, wherein the image module is a photosensor.

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30. The method of claim 29, wherein the photosensor is stationary.

31. The method of claim 26, wherein the at least one image is acquired using an image device; and
wherein the step of acquiring at least one image comprises acquiring the image
5 without moving the image device and the substrate relative to one another.
32. The method of claim 26, wherein the step of acquiring at least one image comprises acquiring a plurality of images to obtain an optimal image.
- 10 33. The method of claim 26, wherein the step of correcting at least one type of distortion comprises correction of grayscale distortion.
34. The method of claim 33, wherein the at least one image is acquired using an image device with a field of view; and
15 wherein the correction of grayscale distortion comprises applying a compensation model for brightness across the field of view for the image device.
35. The method of claim 34, wherein the compensation model is derived by acquiring images using a consistent light source at different brightness values and by
20 using a calibrated set of filters to generate curves for the images acquired at the different brightness values.
36. The method of claim 26, wherein the step of correcting at least one type of distortion comprises correction of spatial distortion.
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37. The method of claim 36, wherein the correction of spatial distortion comprises:
generating a plurality of points distorted by the spatial distortion;
generating a plurality of points undistorted by spatial distortion;
30 generating a model based on the plurality of distorted and undistorted points;
and

applying the model to the image acquired.

38. The method of claim 26, further comprising the step of performing adaptive thresholding on at least a portion of the image acquired.